Title: Imaging Oxygen Concentrations in Bone Scaffolds During Cellular Activity and Fluid Perfusion

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Abstract
Developing bone replacement scaffolds has been a driving ambition of regenerative medicine. Although great progress has been achieved for small scaffolds, the real clinical need is for large scaffolds (>5 mm). Oxygenating these scaffolds is challenging as slow diffusion rates typically lead to necrotic regions in the scaffold core. Here, we study scaffold oxygenation *in vitro* using external perfusion pumps while imaging oxygen concentrations below the scaffolds. We demonstrate that without perfusion, yeast cells growing in the scaffold deplete oxygen, especially from the scaffold center, thus creating necrotic conditions in the scaffold core. The oxygen is restored via pumping fresh medium through the scaffold. The oxygen profiles are highly reproducible from cycle to cycle and both the oxygen dynamics and stationary distributions follow accurately diffusion-reaction models. This lays the groundwork for future *in vivo* oxygen imaging studies using localized light sources and external perfusion pumps for modulation.